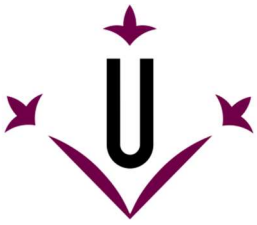

Title: Subcutaneous advanced glycation end-products and lung function according to glucose abnormalities: The ILERVAS Project

Authors: Enric Sánchez Albert Lecube Àngels Betriu Cristina Hernández Carolina López-Cano Liliana Gutiérrez-Carrasquilla Mohsen Kerkeni Andree Yeramian Francesc Purroy Reinald Pamplona Cristina Farràs Elvira Fernández Ferrán Barbé Rafael Simó, on behalf of the ILERVAS project

PII: S1262-3636(18)30079-X
DOI: https://doi.org/doi:10.1016/j.diabet.2018.04.002
Reference: DIABET 1002

To appear in: *Diabetes & Metabolism*

Received date: 14-3-2018
Accepted date: 10-4-2018

Please cite this article as: Sánchez E, Lecube A, Betriu À, Hernández C, López-Cano C, Gutiérrez-Carrasquilla L, Kerkeni M, Yeramian A, Purroy F, Pamplona R, Farràs C, Fernández E, Barbé F, Simó R, on behalf of the ILERVAS project, Subcutaneous advanced glycation end-products and lung function according to glucose abnormalities: The ILERVAS Project, *Diabetes and Metabolism* (2018), https://doi.org/10.1016/j.diabet.2018.04.002

Subcutaneous advanced glycation end-products and lung function according to glucose abnormalities: The ILERVAS Project

Enric Sánchez [1 *], Albert Lecube [1, 2 *], Àngels Betriu [3], Cristina Hernández [2, 4], Carolina López-Cano [1], Liliana Gutiérrez-Carrasquilla [1], Mohsen Kerkeni [5], Andree Yeramian [1], Francesc Purroy [6], Reinald Pamplona [7], Cristina Farràs [8], Elvira Fernández [3], Ferrán Barbé [9, 10], and Rafael Simó [2, 4] on behalf of the ILERVAS project

* E.S. and A.L. contributed equally to this work.

ILERVAS Project: Marta Hernández [1, 2], Ferran Rius [1, 2], Dinora Polanco [9], Manuel Sánchez de la Torre [9, 10], Gerard Torres [9, 10], Pere Godoy [9, 10], Manuel Portero-Otin [7], Mariona Jové [7], Laura Colàs-Compàs [6], Ikram Benabdelhak [6], Eva Miquel [8], Marta Ortega [8], José Manuel Valdivielso [3], Marcelino Beramúdez [3], Montse Martínez-Alonso [3]

[1] Endocrinology and Nutrition Department, University Hospital Arnau de Vilanova Obesity, Diabetes and Metabolism research group, IRBLleida, University of Lleida, Spain

[2] Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

[3] Unit for the Detection and Treatment of Atherothrombotic Diseases (UDETMA V&R), University Hospital Arnau de Vilanova Vascular and Renal Translational Research Group, IRBLleida, University of Lleida, Spain

[4] Endocrinology and Nutrition Department, University Hospital Vall d'Hebron Diabetes and Metabolism Research Unit, VHIR, Autonomous University of Barcelona, Spain

[5] Higher Institute of Biotechnology, Laboratory of Biochemistry, Faculty of Medicine, University of Monastir, Monastir, Tunisia

[6] Stroke Unit, University Hospital Arnau de Vilanova Clinical Neurosciences Group, IRBLleida, University of Lleida, Spain

[7] Metabolic Pathophysiology Group, IRBLleida, University of Lleida, Spain

[8] Borges Blanques Primary Health Care Unit, Spain

[9] Respiratory Department, University Hospital Arnau de Vilanova-Santa María Translational Research in Respiratory Medicine, IRBLleida, University of Lleida, University Hospital Arnau de Vilanova, Spain

[10] Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Received 14 March 2018; Accepted 10 April 2018

Corresponding authors:

Albert Lecube M.D., Ph.D
Endocrinology and Nutrition Department
Hospital Universitari Arnau de Vilanova, Institut de Recerca Biomèdica de Lleida
Avda Rovira Roure 80, 25198 Lleida, Spain
Tel: +34 973 70 51 83; Fax: +34 973 70 51 89
Email: alecube@gmail.com

Rafael Simó M.D., Ph.D
Endocrinology and Nutrition Department, Hospital Universitari Vall d'Hebron Diabetes and Metabolism Research Unit, Vall d'Hebron Institut de Recerca
Pg Vall d'Hebron 119-129, 08024 Barcelona, Spain
Tel: +34 93; Fax: +34 93
Email: rafael.simo@vhebron.net

Keywords: Advanced glycation end-products; Pulmonary function; Skin autofluorescence; Spirometry; Type 2 diabetes

The lung is not usually included in the list of organs that might be affected by type 2 diabetes (T2D). However, its abundance of collagen and elastin fibres, crucial proteins in the extracellular matrix, together with its vascularization reach, make the lung parenchyma a potential target for chronic hyperglycaemia [1]. Indeed, cross-sectional studies conducted during the past few decades have shown that adults with T2D have lower forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) than adults without T2D [2]. A few pathophysiological mechanisms have also been well documented, including insulin and leptin resistance, low-grade chronic inflammatory status, microvascular lung damage and autonomic neuropathy [1].

However, little is known of the potential relationship between advanced glycation end-products (AGEs) and lung function, and what scarce information there is has been focused on patients with chronic obstructive pulmonary disease (COPD), in which higher skin AGE deposition and plasma AGE concentrations have been reported [3]. Yet, the relationship between AGEs and pulmonary function, taking into account the presence of glucose abnormalities, has not been previously examined. For this reason, skin AGE accumulation and spirometric manoeuvres were assessed in a large population with no known pulmonary disease according to the presence of glucose abnormalities.

Both our control and prediabetes populations were recruited from a total of 1924 Caucasian subjects enrolled between July 2015 and May 2017 into the ILERVAS project (ClinTrials.gov Identifier: NCT03228459). This ongoing randomized interventional study is concerned with early diagnosis of subclinical vascular and 'hidden' kidney diseases [4]. Inclusion criteria were: age between 45–70 years; no history of cardiovascular disease or T2D; and at least one cardiovascular risk factor (obesity, hypertension, dyslipidaemia, smoking or first-degree relative with premature cardiovascular disease). Exclusion criteria were: COPD; T2D; chronic kidney disease; active neoplasia; life expectancy < 18 months; pregnancy; and darker skin colour (Fitzpatrick scale types > 5). Smokers who had stopped smoking ≥ 1 year prior to recruitment were considered former smokers. Prediabetes was diagnosed in 34.6% (n = 660) of subjects according to American Diabetes Association criteria [glycosylated haemoglobin (HbA1c): 39–47 mmol/mol or 5.7–6.4%]. Also, 79 age-matched T2D patients

were recruited from the outpatients diabetic clinic of University Hospital Arnau de Vilanova in July 2017. Informed consent was obtained from all participants, and the protocol was approved by the Arnau de Vilanova University Hospital ethics committee.

Anthropometric data were obtained by standardized protocols. Glycosylated haemoglobin was determined using the cobas b 101® system (Roche Diagnostics International, Rotkreuz, Switzerland). Dry samples of capillary blood were used for analyses of values of total cholesterol and serum creatinine.

Forced spirometry was performed using a portable ultrasonic spirometer (Sibelmed DATOSPIR, Sibel S.A., Barcelona, Spain). Subjects were required to have at least three reproducible measurements, and the output with the highest total FEV1 and FVC scores was used for analyses. Various spirometric parameters were measured as a percentage of predicted values. 'Normal' FEV1 was defined as a value ≥ 80% of that predicted, a 'non-obstructive ventilatory defect' as an FVC < 80% of predicted value with an FEV1/FVC ratio ≥ 70%, and an 'obstructive ventilatory defect' as an FEV1/FVC ratio < 70%, as per criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD).

Skin autofluorescence (AF) was measured on the left forearm using the AGE Reader™ (DiagnOptics, Groningen, Netherlands), a fully automated, non-invasive, non-operator-dependent desktop device that uses ultraviolet A (UVA) spectrum wavelengths. The mean AF value of three readings, expressed in arbitrary units (AU), was recorded. The same device was used for measurements in all participants.

Normally distributed variables were evaluated using the Shapiro–Wilk test. Given their skewed distribution, quantitative data were expressed as medians (interquartile range, IQR). Comparisons between groups were made using the Mann–Whitney U test or Pearson's chi-squared test, and the relationship between continuous variables was assessed by Spearman's correlation test.

Accuracy of skin AF as a measurement of interest to discriminate patients with FEV1 scores ≤ 80% of predicted from normal cases was evaluated using receiver operating characteristic (ROC) curve analysis and a complete sensitivity/specificity report. In addition, comparisons between area under the ROC (AUROC) of skin AF and HbA1c were performed using the Hanley and McNeil test.

Stepwise multivariate regression analysis explored the variables independently associated with FVC and FEV1, including age, gender, body mass index (BMI), tobacco use (number of pack-years), HbA1c and skin AF. Statistical significance was accepted at the level of $P < 0.05$. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 20.0, software (IBM Corp., Armonk, NY, USA).

The main clinical characteristics of the study population are shown in Table I. Skin AF was significantly higher in patients with T2D than in those with either prediabetes [2.5 (2.0–3.0) *vs* 1.9 (1.7–2.2) AU; $P < 0.001$] or no glucose abnormalities [1.9 (1.7–2.2) AU; $P < 0.001$]. No differences in skin AF levels were observed between the latter two groups. In addition, patients with T2D showed significantly lower FEV1 and FVC scores, and a greater prevalence of FEV1 scores < 80% than either prediabetes or non-diabetes subjects (Table S1; see supplementary materials associated with this article online). Those with prediabetes showed significantly lower FVC scores than those with no glucose abnormalities ($P = 0.046$).

When skin AGE deposition was evaluated according to ventilatory pattern, patients with respiratory (non-obstructive or obstructive) defects exhibited significantly increased skin AF in comparison to subjects with normal pulmonary function (all $P < 0.001$). When the entire study population was evaluated, positive correlations were found between skin AF and age, HbA1c, tobacco packs/year and estimated glomerular filtration rate (eGFR) on univariate analysis (Table S2; see supplementary materials associated with this article online). In addition, a significant but negative correlation between skin AF and pulmonary parameters, such as FVC ($r = -0.114$, $P < 0.001$) and FEV1 ($r = -0.212$, $P < 0.001$), was observed. Moreover, these correlations became even stronger when only T2D patients were analyzed (FVC: $r = -0.453$, $P < 0.001$; FEV1: $r = -0.393$, $P < 0.001$).

ROC analysis revealed that the optimal cut-off point for skin AF was 2.05 AU: at this point, the AUROC was 0.614 (0.582–0.646), with a sensitivity of 53.5% and specificity of 63.3% (Fig. 1). The percentage of subjects with FEV1 < 80% increased from 17.4% in those with skin AF < 2.05 AU to 27.6% in those with skin AF ≥ 2.05 AU ($P < 0.001$). These data indicate a twofold greater risk of having an abnormal FEV1 (mean difference: 1.9, 95% CI: 1.5–2.3; $P < 0.001$) compared with subjects with lower skin AF values**.** In addition, skin AF significantly improved the AUROC curve obtained with HbA1c measurement [0.614

(0.582–0.646) *vs* 0.555 (0.521–0.589); *P* = 0.014]. Furthermore, stepwise multivariate regression analysis revealed that skin AF (along with gender, BMI, tobacco use and HbA1c) was independently associated with predicted measures of FEV1 (R^2 = 0.121) and FVC (R^2 = 0.122) (Table S3; see supplementary materials associated with this article online).

To the best of our knowledge, this is the first-ever study of subjects without pulmonary disease to demonstrate that skin AGE deposition is related to a decrease in spirometric values and a larger percentage of abnormal ventilatory patterns. In addition, this negative association was more aggravated among patients with T2D.

AGE formation increases with age and is accelerated by chronic hyperglycaemia, chronic inflammation and oxidative stress [5]. Skin AF is also associated with several clinical variables (for example, age, creatinine clearance), lifestyle factors (smoking status, coffee consumption), and genetic polymorphisms [6]. Our present data from a large population are in concordance with those findings. As AGEs are mainly irreversibly linked to tissue proteins, its accumulation is greater with slow turnover rates, as seen in subcutaneous tissue, lens and cartilage [5,6].

Similar to other cross-sectional studies, our present study has shown how patients with T2D exhibit a 13% decrease in FEV1 and 14% decrease in FVC of a theoretical value compared with non-diabetes subjects [1, 2]. On this basis, it has been suggested that non-enzymatic glycosylation of pulmonary parenchymal proteins and chest wall cartilage may favour the development of less-compliant lung parenchyma and, thus, limit chest mobility, an underlying factor in the restrictive respiratory pattern described in T2D [1]. Our results, with skin AF as an independent risk factor for spirometric values, support the potentially deleterious impact of AGEs on pulmonary function.

The role of AGEs in the genesis and rapid progression of both macro- and microvascular chronic T2D complications has been suggested previously [7]. Likewise, descriptions of messenger RNA (mRNA) expression of AGE receptor (RAGE) by type-II alveolar epithelial cells are worthy of attention [8]. Indeed, AGE–RAGE interactions in the lungs might trigger pathophysiological cascades, leading to impaired pulmonary function through lung endothelial cell

dysfunction, proinflammatory effects and cell apoptosis [8]. It is also worth mentioning that our data revealed that prediabetes has a negative impact on FVC in comparison to subjects with normal glucose metabolism but similar skin AF. This finding suggests that mechanisms (such as insulin resistance) other than AGE accumulation might be playing a primary role in initiating the lung impairment seen in T2D.

The involvement of AGEs in patients with COPD has also been previously evaluated, and skin AF was significantly higher in 202 patients with mild-to-very-severe COPD compared with 193 old and young healthy controls [3]. Similar results were also observed in a smaller group of patients, with skin AF proving to be a negative determinant of FEV1 even after adjusting for age, gender and pack-years of smoking [9]. In fact, our present study demonstrates that the negative correlation between skin AF and pulmonary function is not restricted to COPD, as the association was stronger among patients with T2D than in non-diabetes subjects.

Nevertheless, our study has a few limitations. First, it would have been of interest to compare skin AF data with plasma AGE concentrations. Second, as skin AF mainly provides information on AGEs linked to fluorescent proteins, the role of other compounds was not evaluated and, third, the cross-sectional nature of the study does not permit causality to be established.

In conclusion, the present study has provided the first clinical evidence that, in people with no known pulmonary disease, AGEs, as measured by skin AF, are correlated with a decline in spirometric values. The link was even stronger among patients with T2D, suggesting that the accumulation of AGEs in lung parenchyma and the chest wall may now be added to the mechanisms involved in the deleterious effects of T2D on lung function.

Disclosure: The authors report no conflicts of interest in this work.

Acknowledgements

This work was supported by grants from the Lleida Provincial Council, Renal Foundation Jaume Arnó, ISCIII (PI 12/00803 and PI 15/00260) and European Union [European Regional Development Fund (Fondo Europeo de Desarrollo Regional, Una manera de hacer Europa)]. CIBER de Diabetes y Enfermedades Metabólicas Asociadas and CIBER de Enfermedades Respiratorias are initiatives of the Instituto Carlos III. These funders had no role in the study design, data collection or analysis, the decision to publish or preparation of the manuscript. Also, the authors would like to thank Eva Castro, Virtudes María, Teresa Molí, Teresa Vidal, Cristina Dominguez, Marta Elias, Núria Sans and Meritxell Soria for their efforts in the accurate development of the ILERVAS project.

Appendix supplementary material

Supplementary materials (Tables S1–S3) associated with this article can be found at http://www.scincedirect.com at doi . . .

Figure legend

Fig. 1. Receiver operating characteristic (ROC) curve analysis was used to evaluate the accuracy of HbA1c and skin autofluorescence (AF) as a measurement of interest to discriminate between disease cases [patients with forced expiratory volume in the first second (FEV1) < 80% of predicted value] and normal cases, together with a sensitivity/specificity report for the entire study population. Total area under the ROC curve value was interpreted as: 0.9–1.0, excellent; 0.8–0.9, good; 0.7–0.8, fair; 0.6–0.7, poor; and 0.5–0.6, not useful.

References

1. Lecube A, Simó R, Pallayova M, Punjabi NM, López-Cano C, Turino C, et al. Pulmonary function and sleep breathing: two new targets for type 2 diabetes. Endocr Rev 2017; 38: 550-73.

2. van den Borst B, Gosker HR, Zeegers MP, Schols AMWJ. Pulmonary function in diabetes. A metaanalysis. Chest 2010; 138: 393–406.

3. Hoonhorst SJ, Lo Tam Loi AT, Hartman JE, Telenga ED, van den Berge M, Koenderman L et al. Advanced glycation end products in the skin are enhanced in COPD. Metabolism 2014; 63:1149-56.

4. Betriu À, Farràs C, Abajo M, Martínez-Alonso M, Arroyo D, Barbe F et. al. Randomised intervention study to assess the prevalence of subclinical vascular disease and hidden kidney disease and its impact on morbidity and mortality: The ILERVAS project. Nefrologia 2016: 36: 389-96.

5. Ajith TA, Vinodkumar P. Advanced glycation end products: association with the pathogenesis of diseases and the current therapeutic approaches. Curr Clin Pharmacol 2016; 11: 118-27.

6. van Waateringe RP, Slagter SN, van der Klauw MM, van Vliet-Ostaptchouk JV, Graaff R, Paterson AD, et al. Lifestyle and clinical determinants of skin autofluorescence in a population-based cohort study. Eur J Clin Invest 2016; 46:481-90.

7. Bentata R, Cougnard-Grégoire A, Delyfer MN, Delcourt C, Blanco L, Pupier E, et al. Skin autofluorescence, renal insufficiency and retinopathy in patients with type 2 diabetes. J Diabetes Complications 2017; 31: 619-23.

8. Ota C, Ishizawa K, Yamada M, Tando Y, He M, Takahashi T, et al. Receptor for advanced glycation end products expressed on alveolar epithelial cells is the main target for hyperoxia-induced lung injury. Respir Investig 2016; 54: 98–108.

9. Hoonhorst SJ, Lo Tam Loi AT, Pouwels SD, Faiz A, Telenga ED, van den Berge M. Advanced glycation end-products and their receptor in different body compartments in COPD. Respir Res 2016; 17: 46.

Table I. Main clinical characteristics, metabolic data, pulmonary function and breathing pattern parameters in the study population according to glucose abnormalities

	T2D patients	**Prediabetes**	**Non-T2D patients**
n	79	660	1170
Women, n (%)	39 (49.4)	393 (59.5)	567 (48.5)
Age (years)	61 (55–65)	59 (54–64)	57 (52–62)
Known T2D duration (years)	12 (8–15)	–	–
Body mass index (kg/m^2)	30.8 (27.8–35.6)	29.6 (26.9–33.2)	28.1 (25.3–31.4)
Current smoker, n (%)	18 (22.7)	155 (23.4)	387 (33.0)
Tobacco (n, pack-years)	21.0 (15.0–42.8)	20.9 (10.1–34.8)	20.5 (9.0–32.0)
HbA1c (%)	8.4 (6.7–9.6)	5.8 (5.7–6.0)	5.4 (5.2–5.5)
HbA1c (mmol/mol)	67 (50–81)	40 (39–42)	36 (33–37)
Total cholesterol (mg/dL)	185 (159–213)	203 (182–229)	201 (180–227)
eGFR (mL/min/1.73 m^2)	90.0 (74.7–94.9)	95.8 (85.9–101.9)	97.1 (87.7–103.3)
Skin autofluorescence (AU)	2.5 (2.0–3.0)	1.9 (1.7–2.2)	1.9 (1.7–2.2)

Data are expressed as medians (IQR) or as n (%);

T2D: type 2 diabetes; eGFR: estimated glomerular filtration rate by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

Table S1. Main pulmonary function parameters and breathing patterns in the study population according to glucose abnormalities (GAs)

	T2D patients	With GAs	Without GAs	*P**	*P***
n	79	660	1170	–	–
FEV1 (% predicted)	82 (74–97)	93 (82–106)	95 (83–106)	< 0.001	< 0.001
FVC (% predicted)	80 (68–91)	92 (81–103)	94 (83–103)	< 0.001	< 0.001
FEV1/FVC	82 (74–85)	79 (75–83)	79 (74–83)	0.061	0.020
FEV1 < 80% predicted, n (%)	35 (44.3)	135 (20.5)	211 (18.0)	< 0.001	< 0.001
Non-obstructive ventilatory defect,[a] n (%)	32 (40.5)	127 (19.2)	186 (15.9)	< 0.001	< 0.001
Obstructive ventilatory pattern[a], n (%)	11 (13.9)	74 (11.2)	129 (11.0)	0.475	0.429

Data are expressed as medians (IQR) or as n (%);
* T2D *vs* prediabetes; ** T2D *vs* normal glucose metabolism;
T2D: type 2 diabetes; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second; [a] as per Global Initiative for Chronic Obstructive Lung Disease (GOLD)

Table S2. Correlation of skin autofluorescence (AF) with clinical characteristics, metabolic data and pulmonary parameters

	Skin AF	
	r	***P***
Age (years)	0.232	< 0.001
T2D duration (years)	-0.120	0.423
Body mass index (kg/m^2)	-0.004	0.854
Tobacco use (n, pack-years)	0.277	< 0.001
HbA1c (% or mmol/mol)	0.086	< 0.001
eGFR (mL/min/1.73 m^2)	-0.156	< 0.001
FEV1 (% predicted)	-0.212	< 0.001
FVC (% predicted)	-0.114	< 0.001
FEV1/FVC	-0.103	< 0.001

T2D: type 2 diabetes; HbA1c: glycosylated haemoglobin; eGFR: estimated glomerular filtration rate by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second

Table S3. Stepwise multivariate regression analysis of variables associated with forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1)

		β	B (95% CI)	*P*
FEV_1 (% predicted)	Gender (male/female)	0.139	4.944 (2.968 to 6.950)	< 0.001
	HbA1c (%)	-0.092	-2.675 (-4.295 to -1.056)	0.001
	Skin autofluorescence (AU)	-0.102	-3.739 (-5.875 to -1.602)	0.001
	Body mass index (kg/m^2)	-0.116	-0.400 (-0.591 to -0.209)	< 0.001
	Tobacco use (n, packs-year)	-0.200	-0.195 (-0.252 to -0.138)	< 0.001
	Age (years)	-0.027	–	0.356
R^2 = 0.121	*Constant*	–	–	< 0.001
FVC (% predicted)	Gender (male/female)	0.180	6.231 (4.276 to 8.187)	< 0.001
	Skin autofluorescence (AU)	-0.076	-2.741 (-4.823 to -0.659)	0.010
	HbA1c (%)	-0.103	-2.923 (-4.501 to -1.344)	< 0.001
	Tobacco use (n, packs-year)	-0.117	-0.111 (-0.166 to -0.056)	< 0.001
	Body mass index (kg/m^2)	-0.187	-0.630 (-0.816 to -0.443)	< 0.001
	Age (years)	-0.054	–	0.061
R^2 = 0.122	*Constant*	–	–	< 0.001

HbA1c: glycosylated haemoglobin